receptor ($GABA_AR$). Super-resolution techniques [2] based on Individual Molecule Localization (IML) [3] provide the capability to quantitatively study these subcellular structures, giving access to its protein distribution at the molecular level.
In this work the highly heterogeneous spatial distribution of Gephyrin cluster along the 3D neuronal network has been investigated through dual color STORM (*Stochastic Optical Reconstruction Microscopy*) to characterize the post-synaptic area, for example reveling the interaction between Gephyrin and $GABA_ARs$.
To monitor the response of Gephyrin cluster to Long-Term Potentiation of inhibition (iLTP) their functional link with the synaptic terminals has been highlighted. The insight provided at the molecular level makes IML techniques the suitable tool for a quantitative study of Gephyrin distribution at synaptic level. Quantitative approach based on clustering analysis [4] provided access to a more comprehensive set of parameters able to define the response to iLTP, such as the area and the density of the scaffold protein. At the fluorescent tag level, to fulfill the requests of the quantitative analysis, an irreversibly photoactivatable fluorescent protein has been used (mEos), knowing its photophysical properties [5].
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Single Molecule Analysis of Endogenous mRNA in Stress Granules
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In eukaryotic cells, mRNA is transcribed from DNA in the nucleus, and conveys genetic information to the ribosome in the cytoplasm. There are various regulation steps in the life of mRNA, including transcription, translation and degradation. When cells are exposed to environmental stresses, cytoplasmic mRNA and several proteins affecting mRNA functions assemble and form densely packed granular structures, which are called stress granule (SG). Previous studies showed that SG play an important role in mRNA remodeling for translational repression, but the underlying mechanism remains unclear. This is partly because there has been no method to visualize and analyze the spatiotemporal regulation of endogenous mRNA with sufficient performance in highly dense environments like SGs. Here, we investigated the behavior of endogenous mRNA in SG at the single-molecule level by using super-resolution localization microscopy and single-particle tracking. Super-resolution imaging revealed that mRNA was distributed heterogeneously in SG, whereas it was elusive in conventional fluorescence imaging. The results obtained by single-particle tracking represent that mRNA motility was suppressed inside SG compared to outside SG. These results suggest that SG has highly organized composition that might be responsible for the physiological functions in cellular stress responses. Therefore, the combination of super-resolution imaging and single particle tracking is a powerful tool to investigate the detailed organization of densely packed granular architectures in cells.

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Super-Oscillatory Imaging of Nanoparticle Interactions with Neurons
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We are developing a super-resolution imaging technology, called the Nanoscope, to allow label-free imaging of sub-cellular processes in living neural cells at nanometre resolution. This technique is an enhancement to current confocal microscope technologies and, unlike most super-resolution technologies, does not rely on fluorescent labelling. The phenomenon of super-oscillation means that by precisely controlling the interference of many band-limited waves, it is possible to generate a local region containing very high spatial frequencies. In the context of optics, this allows us to break the diffraction limit by making arbitrarily small spots far from any lens, within a dark field-of-view, surrounded by sidebands - trading efficiency for resolution. We select scattered light from the central spot using conventional optics, eliminating the sidebands and achieving resolution determined by central spot size. Our approach replaces a conventional microscope objective with a 'super-oscillatory lens' which sculpts the input light to form our strongly-confined spot. We have two methods of achieving this: the first uses a carefully designed set of concentric metal rings milled on a glass substrate to focus the light directly. The second method uses a spatial light modulator to structure the beam incident on a standard objective, shaping the standard focal spot into a sub-wavelength super-oscillatory spot.
We will use the super-oscillatory microscope to further the understanding of cellular function, during health and neurodegenerative diseases. Our initial experiments utilise unlabelled gold nanorods (500x75nm) in low-density primary neuronal cultures. Localisation and tracking of individual gold nanoparticles will allow us to determine the effect of nanoparticles on cell function, mechanisms of uptake and potential clearing. This technique can also be used to investigate the general mechanisms of uptake and subcellular trafficking, providing information on the cell's essential abilities to dynamically compartmentalise materials within the neuronal architecture.

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Quantitative Super-Resolution Microscopy using Novel Meditope Reagents
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Pointillistic super-resolution microscopy techniques are excellent tools for quantitative interrogation of protein distribution and dynamics on a nanoscale level. To efficiently detect single molecules, proteins of interest need to be genetically tagged with optical highlighter proteins or affinity tagged with antibodies labeled with fluorescent (or caged) dyes. We leveraged advantages of both approaches by utilizing a unique peptide-binding site in the Fab framework of monoclonal antibody: the meditope [1]. In this manner we can achieve stoichiometric and site-specific labeling of endogenous proteins using meditope-enabled antibodies.
We coupled a high-affinity meditope to a series of optical highlighter proteins to perform advanced photo-activated localization microscopy (PALM) imaging of human epidermal growth factor receptor 2 (Her2) on the membrane of breast cancer cell lines SK-BR-3 and BT-474. We first characterized binding and we typically obtain 10 nm resolution. Next, we used pair-correlation analysis [2] to quantitatively investigate distribution of Her2. While imaging with Fab complexes (one fluorophore/Fab) results in random distribution of Her2, imaging with Ab complexes (two fluorophores/Ab), results in mostly dimeric distribution of Her2. We anticipate that in the future our approach will provide invaluable insights on molecular dynamics and nanoscale spatial organization of growth factor receptors.
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Fourier Transform Infrared Spectroscopy and Imaging in Cancer Diagnosis and Characterization
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Fourier Transform Infrared (FTIR) spectroscopy and imaging techniques are formidable, novel, rapid and non-destructive tools for characterization of different biological systems from molecules to membranes, cells and tissues. These techniques with multivariate analysis tools can be used in the diagnosis/follow-up of diseases including cancer, and in monitoring drug or chemical induced alterations in tissues and cells. In the current study, Attenuated Total Reflectance FTIR spectroscopy together with chemometric (cluster and principal component) analysis were utilized as a diagnostic tool for urinary bladder cancer while FTIR imaging technique was used in the clarification of sodium butyrate (NaB) induced-differentiation in colon cancer cells since butyrate has an anti-proliferative effect in colon cancer. For bladder cancer studies, bladder wash samples of bladder cancer and control groups were used directly for spectra collection. ATR-FTIR studies revealed significant alterations in lipid, protein, and nucleic acid content of bladder wash samples of cancer groups as compared to the control ones. Based on these spectral variations, bladder cancer group can be successfully differentiated from control via chemometric analysis with a higher sensitivity and specificity than many other methods used currently in bladder cancer diagnosis. In colon cancer study, aggressive CaCo2 cell lines were treated with 3mM NaB and cultured for 48h for complete differentiation. Specific band ratio analysis such as lipid/protein, glycogen/phosphate and RNA/DNA were calculated from the chemical maps of control